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(71) Applicant *(for all designated States except US)*: CELLTECH THERAPEUTICS LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: DOCHERTY, Andrew, James, Penrose [GB/GB]; Piccards Cottage, Sandy Lane, Guildford, Surrey GU3 1HF (GB). SLOCOMBE, Patrick, Marcel [GB/GB]; 16 Roman Way, Warfield, Bracknell, Berkshire RG42 7UT (GB).

(74) Agent: HALLYBONE, Huw, George; Carpmaels & Ransford, 43 Bloomsbury Square, London, WC1A 2RA (GB).

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(54) Title: DNA SEQUENCES CODING FOR A HUMAN METALLOPROTEINASE AND VARIANTS THEREOF

(57) Abstract

DNA sequences coding for a human metalloproteinase are described together with the corresponding antisense DNA and RNA. The DNA may be used to produce the metalloproteinase which may be used to generate antibodies thereto and to obtain other compounds capable of regulating the action of the metalloproteinase *in vivo*.

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DNA SEQUENCES CODING FOR A HUMAN METALLOPROTEINASE AND VARIANTS THEREOF

This invention relates to a novel human metalloproteinase, to homologues and fragments thereof, to means for producing the metalloproteinases, and to means for regulating their production and activity *in vivo*.

A number of physiologically important processing events are mediated by metalloproteinases, which under certain circumstances contribute to pathologies as diverse as inflammation and cancer, and it has been suggested that such enzymes would provide targets for therapeutic intervention. Thus, by varying the production of the enzyme, or inhibiting or enhancing its activity *in vivo* it should be possible to achieve a therapeutic effect.

In one example, tumour necrosis factor-alpha (TNF-α) is a potent pro-inflammatory and immunomodulatory mammalian cytokine produced primarily by activated monocytes and macrophages. It is initially expressed as a 233-amino-acid membrane-anchored precursor (pro-TNF-α) which is proteolytically processed to yield the mature, 157-amino-acid cytokine. Evidence has been obtained which indicates that at least one metalloproteinase-like enzyme mediates pro-TNF-α cleavage, but to date the enzyme(s) responsible for this *in vivo* are unknown [see for example Mohler, K M *etal*, Nature 370, 218-220 (1994); Gearing, A J *et al ibid* 370, 555-557 (1994); McGeehan, G M *et al*, *ibid* 370, 558-561 (1994)]. A number of known matrix metalloproteinase inhibitors have been shown to block TNF-α secretion [see the above papers and International Patent Specification Publication No. WO 95/06031]. These compounds were originally designed to selectively inhibit matrix metalloproteinases such as collagenase with primary functions unrelated to pro-TNF-α cleavage. Where new inhibitors have been described these have apparently been selected on the basis of their effect on TNF-α secretion seen in cell-based assays.

In another example, L-selectin shedding is thought to be a pro-inflammatory event that is mediated by an as yet unidentified metalloproteinase [Lasky, Science, 258, 964-969 (1992)]. Some inhibitors of L-selectin proteolysis have been identified, but these have been obtained using cell based assays [Walchech *et al.*, Nature, 380, 720-723 (1996); Feehan *et al.*, J. Biol. Chem., 271, 7019-7024 (1996)].

In general, in order to obtain compounds capable of selectively regulating the action of a metalloproteinase implicated in human disease, for example as in the above TNF-α and L-selectin instances it would be clearly advantageous to have the enzyme unequivocally identified and obtainable in an isolated, purified and unambiguous characterised form.

Through the use of a cloning and screening approach, we have been able to identify human DNA which is responsible for coding part of one such metalloproteinase. This DNA has the sequence described in SEQ I.D. No: 1 below and may be of use (1) in the generation of a gene coding for the metalloproteinase, (2) in the production of the metalloproteinase, (3) in the provision of means to regulate the activity of the metalloproteinase *in vivo*, and (4) in the provision of means to detect and measure a metalloproteinase in a biological system, e.g. in serum, synovial fluid or a tissue extract.

Thus according to one aspect of the invention we provide DNA comprising the nucleotide sequence of SEQ I.D. No: 1:

SEQ I.D. No: 1

GGTTCGAGCACTCCAAGCCCACCACCAGGGACTGGGCTCTTCAGTTT
ACACAACAGACCAAGAAGCGACCTCGCAGGATGAAAAGGGAAGATTT
AAACTCCATGAAGTATGTGGAGCTTTACCTCGTGGCTGATTATTTAGA
GTTTCAGAAGAATCGACGAGACCAGGACGCCACCAAACACAAGCTCA
TAGAGATCGCCAACTATGTTGATAAGTTTTACCGATCCTTGAACATCC
GGATTGCTCTCGTGGGCTTGGAAGTGTGGACCCACGGGAACATGTG
TGAAGTTTCAGAGAATCCATATTCTACCCTCTGGTCCTTTCTCAGTTG
GAGGCGCAAGCTGCTTGCCCAGAAGTACCATGACAACGCCCAATTAA

TCACGGGCATGTCCTTCCACGGCACCACCATCGGCCTGGCCCCCCT
CATGGCCATGTGCTCTGTGTACCAGTCTGGAGGAGTCAACATGGACC
ACTCCGAGAATGCCATTGGCGTGGCTGCCACCATGGCCCACGAGAT
GGGCCACAACTTTGGCATGACCCATGATTCTGCAGATTGCTGCTCGG
CCAGTGCGGCTGATGGTGGGTGCATCATGGCAGCTGCCACTGGGCA
CCCCTTTCCCAAAGTGTTCAATGGATGCAACAGGAGGGAGCTGGACA
GGTATCTGCAGTCAGGTGGTGGAATGTGTCTCTCCAACATGCCAGAC
ACCAGGATGTTGTATGGAGGCCGGAGGTGTGGGAACGGGTATCTGG
AAGATGGGGAAGAGTGTGACTGTGGAGAAGAAGAGGAATGTAACAA
CCCCTGCTGCAATGCCTCTAATTGTACCCTGAGGCCGGGGGCGGAG
TGTGCTCACGGCTCCTGCTGCCACCAGTGTAAGCTGTTGGCTCCTGG
GACCCTGTGCCGCGAGCAGGCCAGGCAGTGTGACCTCCCGGAGTTC
TGTACGGGCAAGTCTCCCCACTGCCCTACCAACTTCTACCAGATGGA
TGGTACCCCCTGTGAGGGCGGCCAGGCCTACTGCTACAACGGCATG
TGCCTCACCTACCAGGAGCAGTGCCAGCAGCTGTGGGGACCCGGAG
CCCGACCTGCCCCTGACCTCTGCTTCGAGAAGGTGAATGTGGCAGG
AGACACCTTTGGAAACTGTGGAAAGGACATGAATGGTGAACACAGGA
AGTGCAACATGAGAGATGCGAAGTGTGGGAAGATCCAGTGTCAGAG
CTCTGAGGCCCGGCCCCTGGAGTCCAACGCGGTGCCCATTGACACC
ACTATCATCATGAATGGGAGGCAGATCCAGTGCCGGGGCACCCACG
TCTACCGAGGTCCTGAGGAGGAGGGTGACATGCTGGACCCAGGGCT
GGTGATGACTGGAACCAAGTGTGGCTACAACCATATTTGCTTTGAGG
GGCAGTGCAGGAACACCTCCTTCTTTGAAACTGAAGGCTGTGGGAAG
AAGTGCAATGGCCATGGGGTCTGTAACAACAACCAGAACTGCCACTG
CCTGCCGGGCTGGGCCCCGCCCTTCTGCAACACACCGGGCCACGG
GGGCAGTATCGACAGTGGGCCTATGCCCCCTGAGAGTGTGGGTCCT
GTGGTAGCTGGAGTGTTGGTGGCCATCTTGGTGCTGGCGGTCCTCA
TGCTGATGTACTACTGCTGCAGACAGAACAACAAACTAGGCCAACTC
AAGCCCTCAGCTCTCCCTTCCAAGCTGAGGCAACAGTTCAGTTGTCC
CTTCAGGGTTTCTCAGAACAGCGGGACTGGTCATGCCAACCCAACTT
TCAAGCTGCAGACGCCCCAGGGCAAGCGAAAGGTGTTCCTTGACTT
GTGCGTACAGGTGATCAACACTCCGGAAATCCTGCGGAAGCCCTCC
CAGCCTCCTCCCCGGCCCCCTCCAGATTATCTGCGTGGTGGGTCCC
CACCTGCACCACTGCCAGCTCACCTGAGCAGGGCTGCTAGGAACTC
CCCAGGGCCCGGGTCTCAAATAGAGAGGACGGAGTCGTCCAGGAG
GCCTCCTCCAAGCCGGCCAATTCCCCCCGCACCAAATTGCATCGTTT

CCCAGGACTTCTCCAGGCCTCGGCCGCCCCAGAAGGCACTCCCGGC
AAACCCAGTGCCAGGCCGCAGGAGCCTCCCCAGGCCAGGAGGTGC
ATCCCCACTGCGGCCCCCTGGTGCTGGCCCTCAGCAGTCCCGGCCT
CTGGCAGCACTTGCCCCAAAGAGGGTATGGAAGACTTGCAATTTGAA
AACTGGGGACCAGTTCCAAAGTCAGTAATTGTGTTAACCACGTGTATA
ACAGCTCTGCTGGACACCCAAGAAAGCCATGGGAACGCCAACTGGA
AAGGTCCCCTTCCCCAGGGGAGCCTGCGAAGGAGAGGTTCTGTAGA
ATCCAAGCCCACATTTCCAAAGTCACCCCCAACGCGTCCTCTCACAC
CGTCCACTGTGCGTTTGTATGTGTCTGGGATCCAGGGCAATGTGAAT
TTTCTTTTTATTTGGGAGATTGTTCACGGAAAACAGATCTTCTTCTCTC
TTGTCCACCTATTAATTGTTTACAATATTTGTACATCTATGCAAAATAC
TTGAATGGGCCATGGTGCCTTTTTTCCTTGTTAGTATTTAATTAAAAAT
GAATTGTTTGTCATTTGCAAAAAAAAAAAAAAAAAAA

and homologues and fragments thereof.

It will be appreciated that the nucleotide sequence of SEQ I.D. No: 1 also includes control sequences, such as a polyadenylation sequence, providing for expression of the sequence in a host cell.

One particular DNA fragment according to the invention is the isolated human metalloproteinase-encoding nucleotide sequence of SEQ I.D. No: 2:

SEQ I.D. No: 2

TTCGAGCACTCCAAGCCCACCACCAGGGACTGGGCTCTTCAGTTTAC
ACAACAGACCAAGAAGCGACCTCGCAGGATGAAAAGGGAAGATTTAA
ACTCCATGAAGTATGTGGAGCTTTACCTCGTGGCTGATTATTTAGAGT
TTCAGAAGAATCGACGAGACCAGGACGCCACCAAACACAAGCTCATA
GAGATCGCCAACTATGTTGATAAGTTTTACCGATCCTTGAACATCCGG
ATTGCTCTCGTGGGCTTGGAAGTGTGGACCCACGGGAACATGTGTG
AAGTTTCAGAGAATCCATATTCTACCCTCTGGTCCTTTCTCAGTTGGA
GGCGCAAGCTGCTTGCCCAGAAGTACCATGACAACGCCCAATTAATC
ACGGGCATGTCCTTCCACGGCACCACCATCGGCCTGGCCCCCCTCA
TGGCCATGTGCTCTGTGTACCAGTCTGGAGGAGTCAACATGGACCAC

TCCGAGAATGCCATTGGCGTGGCTGCCACCATGGCCCACGAGATGG
GCCACAACTTTGGCATGACCCATGATTCTGCAGATTGCTGCTCGGCC
AGTGCGGCTGATGGTGGGTGCATCATGGCAGCTGCCACTGGGCACC
CCTTTCCCAAAGTGTTCAATGGATGCAACAGGAGGGAGCTGGACAG
GTATCTGCAGTCAGGTGGTGGAATGTGTCTCTCCAACATGCCAGACA
CCAGGATGTTGTATGGAGGCCGGAGGTGTGGGAACGGGTATCTGGA
AGATGGGGAAGAGTGTGACTGTGGAGAAGAAGAGGAATGTAACAAC
CCCTGCTGCAATGCCTCTAATTGTACCCTGAGGCCGGGGGCGGAGT
GTGCTCACGGCTCCTGCTGCCACCAGTGTAAGCTGTTGGCTCCTGG
GACCCTGTGCCGCGAGCAGGCCAGGCAGTGTGACCTCCCGGAGTTC
TGTACGGGCAAGTCTCCCCACTGCCCTACCAACTTCTACCAGATGGA
TGGTACCCCCTGTGAGGGCGGCCAGGCCTACTGCTACAACGGCATG
TGCCTCACCTACCAGGAGCAGTGCCAGCAGCTGTGGGGACCCGGAG
CCCGACCTGCCCCTGACCTCTGCTTCGAGAAGGTGAATGTGGCAGG
GACACCTTTGGAAACTGTGGAAAGGACATGAATGGTGAACACAGGAA
GTGCAACATGAGAGATGCGAAGTGTGGGAAGATCCAGTGTCAGAGC
TCTGAGGCCCGGCCCCTGGAGTCCAACGCGGTGCCCATTGACACCA
CTATCATCATGAATGGGAGGCAGATCCAGTGCCGGGGCACCCACGT
CTACCGAGGTCCTGAGGAGGAGGGTGACATGCTGGACCCAGGGCTG
GTGATGACTGGAACCAAGTGTGGCTACAACCATATTTGCTTTGAGGG
CAGTGCAGGAACACCTCCTTCTTTGAAACTGAAGGCTGTGGGAAGAA
GTGCAATGGCCATGGGGTCTGTAACAACAACCAGAACTGCCACTGCC
TGCCGGGCTGGGCCCCGCCCTTCTGCAACACACCGGGCCACGGGG
GCAGTATCGACAGTGGGCCTATGCCCCCTGAGAGTGTGGGTCCTGT
GGTAGCTGGAGTGTTGGTGGCCATCTTGGTGCTGGCGGTCCTCATG
CTGATGTACTACTGCTGCAGACAGAACAACAAACTAGGCCAACTCAA
GCCCTCAGCTCTCCCTTCCAAGCTGAGGCAACAGTTCAGTTGTCCCT
TCAGGGTTTCTCAGAACAGCGGGACTGGTCATGCCAACCCAACTTTC
AAGCTGCAGACGCCCCAGGGCAAGCGAAAGGTGTTCCTTGACTTGT
GCGTACAGGTGATCAACACTCCGGAAATCCTGCGGAAGCCCTCCCA
GCCTCCTCCCCGGCCCCCTCCAGATTATCTGCGTGGTGGGTCCCCA
CCTGCACCACTGCCAGCTCACCTGAGCAGGGCTGCTAGGAACTCCC
CAGGGCCCGGGTCTCAAATAGAGAGGACGGAGTCGTCCAGGAGGC
CTCCTCCAAGCCGGCCAATTCCCCCCGCACCAAATTGCATCGTTTCC
CAGGACTTCTCCAGGCCTCGGCCGCCCCAGAAGGCACTCCCGGCAA
ACCCAGTGCCAGGCCGCAGGAGCCTCCCCAGGCCAGGAGGTGCAT

CCCCACTGCGGCCCCCTGGTGCTGGCCCTCAGCAGTCCCGGCCTCT
GGCAGCACTTGCCCCAAAGAGGGTATGGAAGACTTGCAATTTGAAAA
CTGGGGACCAGTTCCAAAGTCAG

and homologues and fragments thereof.

In the sequences herein standard one letter codes are used to represent nucleotides or amino acids as appropriate.

DNA according to the invention may be obtained using conventional molecular biology procedures, for example by probing a human genomic or cDNA library with one or more labelled oligonucleotide probes containing for example fifteen or more contiguous nucleotides designed using the nucleotide sequences described herein [see for example "Current Protocols in Molecular Biology", Ausubel, F M *et al* (eds), Greene Publishing Associates and Wiley-Interscience, New York (1987)].

Where the term homologue is used herein in relation to a particular nucleotide or amino acid sequence this is to be understood to represent a corresponding sequence in which one or more nucleotides or amino acids have been added, deleted, substituted or otherwise chemically modified, provided always that the homologue retains substantially the same catalaytic properties as the particular sequence described. One particular type of homologue for example may be that in which one or more nucleotides have been substituted due to the degeneracy of the genetic code. Homologues, particularly longer versions of the sequences described herein, may be obtained by standard molecular biology and/or chemistry techniques, e.g. by cDNA or gene cloning, or by use of oligonucleotide directed mutagenesis or oligonucleotide directed synthesis techniques or enzymatic cleavage or enzymatic filling in of gapped oligonucleotides (see for example Ausubel, F M *ibid*).

The DNA of SEQ I.D. No: 1 and SEQ I.D. No: 2 each codes for part of a human metalloproteinase. Thus, the DNA according to the invention or a fragment thereof may be used as a probe to screen an appropriate genomic or cDNA library in a process utilising standard hybridisation

and/or PCR cloning techniques to obtain the gene or cDNA coding for the entire metalloproteinase, or a homologue or fragment thereof, or a related metalloproteinase from another species.

Thus according to a further aspect of the invention we provide an isolated gene or cDNA coding for a human metalloproteinase, said gene or cDNA containing the nucleotide sequence of SEQ I.D. No: 1 or SEQ I.D. No: 2 or a homologue thereof.

The gene according to the invention may in turn be used to produce a metalloproteinase. In another aspect of the invention we therefore provide an isolated human metalloproteinase which contains the amino acid sequence of SEQ I.D. No: 3:

SEQ I.D. No: 3

FEHSKPTTRDWALQFTQQTKKRPRRMKREDLNSMKYVELYLVADYLEF
QKNRRDQDATKHKLIEIANYVDKFYRSLNIRIALVGLEVWTHGNMCEVSE
NPYSTLWSFLSWRRKLLAQKYHDNAQLITGMSFHGTTIGLAPLMAMCSV
YQSGGVNMDHSENAIGVAATMAHEMGHNFGMTHDSADCCSASAADG
GCIMAAATGHPFPKVFNGCNRRELDRYLQSGGGMCLSNMPDTRMLYG
GRRCGNGYLEDGEECDCGEEEECNNPCCNASNCTLRPGAECAHGSCC
HQCKLLAPGTLCREQARQCDLPEFCTGKSPHCPTNFYQMDGTPCEGG
QAYCYNGMCLTYQEQCQQLWGPGARPAPDLCFEKVNVAGDTFGNCG
KDMNGEHRKCNMRDAKCGKIQCQSSEARPLESNAVPIDTTIIMNGRQIQ
CRGTHVYRGPEEEGDMLDPGLVMTGTKCGYNHICFEGQCRNTSFFETE
GCGKKCNGHGVCNNNQNCHCLPGWAPPFCNTPGHGGSIDSGPMPPE
SVGPVVAGVLVAILVLAVLMLMYYCCRQNNKLGQLKPSALPSKLRQQFS
CPFRVSQNSGTGHANPTFKLQTPQGKRKVFLDLCVQVINTPEILRKPSQ
PPPRPPPDYLRGGSPPAPLPAHLSRAARNSPGPGSQIERTESSRRPPPS
RPIPPAPNCIVSQDFSRPRPPQKALPANPVPGRRSLPRPGGASPLRPPG
AGPQQSRPLAALAPKRVWKTCNLKTGDQFQSQ

and homologues and fragments thereof.

The production of a protein according to the invention may be achieved using standard recombinant DNA techniques involving the expression of the metalloproteinase by a host cell. The isolated nucleic acids described herein may be for example introduced into any suitable expression vector by operatively linking the DNA to any necessary expression control elements therein and transforming any suitable procaryotic or eucaryotic host cell with the vector using well known procedures. The invention is thus to be understood to extend to recombinant plasmids containing a gene of the invention or a nucleotide sequence of SEQ I.D. No: 1 or SEQ I.D. No: 2, to cells containing said recombinant plasmids and to a process for producing the protein according to the invention which comprises culturing said cells such that the desired protein is expressed and recovering the protein from the culture.

Thus in one example the nucleotide sequence of SEQ I.D. No: 1, without its 3' poly A tail, or SEQ I.D. No: 2, or a homologue such as a longer version including a sequence encoding a signal peptide for secretion, and a propeptide to ensure accurate enzyme folding, is inserted downstream of the hCMV promoter in the pEE12 plasmid vector, and either transiently or stably expressed in CHO-L761h or NSO mouse melanoma cells [Murphy et al., J. Biol. Chem., 267, 9612-9618 (1992)]. Expression of the enzyme according to the invention can be detected in serum free culture medium by its catalytic properties, or by Western blotting [Murphy et al., Biochem. J., 283, 637-641 (1992); Murphy et al., J. Biol. Chem., 267, 9612-9618 (1992)]. Such assays can also be used during the subsequent isolation of the expressed enzyme from transfected cell conditioned medium. If the enzyme requires further activation, this may be achieved proteolytically through use of modest amounts of trypsin, furin, or other methods, in order to remove an approximately 180 amino acid N-terminal propeptide, as described for other metalloproteinases [Murphy et al., J. Biol. Chem., 267, 9612-9618 (1992); Crabbe et al., Biochemistry, 33, 14419-14425 (1994); Pei and Weiss, Nature, 375, 244-247 (1995); Will et al., J. Biol. Chem., 271, 17119-17123 (1996)]

It may be desirable to produce the catalytic domain of the protein according to the invention in isolation from the rest of the molecule. This

may be achieved using the above standard recombinant DNA techniques except that in this instance the DNA sequence used is that encoding the amino acid sequence of SEQ I.D. No: 4:

SEQ I.D. No: 4

MKREDLNSMKYVELYLVADYLEFQKNRRDQDATKHKLIEIANYVDKFYR
SLNIRIALVGLEVWTHGNMCEVSENPYSTLWSFLSWRRKLLAQKYHDNA
QLITGMSFHGTTIGLAPLMAMCSVYQSGGVNMDHSENAIGVAATMAHE
MGHNFGMTHDSADCCSASAADGGCIMAAATGHPFPKVFNGCNRRELD
RYLQSGGGMCLSNMPDTRMLYG

or a homologue thereof, and the invention extends to such isolated catalytic domains.

N or C-terminally extended versions of the sequence shown in SEQ I.D. No: 4 may be obtained by expression in procaryotic or eucaryotic cells as described above optionally attached to a peptide tag via which the protein may be affinity purified and identified. Examples of tags include the well known "His" or "Strep-tags". Further sequences that may be attached arise from expression in procaryotic cells and include the pelB or ompA leaders which when placed at the N-terminus help direct secretion to the *E.coli* periplasmic space [Schmidt and Skerra, J. Chromatography, 676, 337-345 (1994); Knauper et al., J. Biol. Chem., 271, 17124-17131 (1996)].

The gene or nucleotide sequences according to the invention may also be of use in diagnosis, for example to determine enzyme deficiency in a human subject, by for example direct DNA sequence comparison or DNA/RNA hybridisation assays; or in therapy, for example where it is desired to modify the production of the metalloproteinase *in vivo*, and the invention extends to such uses.

Knowledge of the gene according to the invention also provides the ability to regulate its activity *in vivo* by for example the use of antisense DNA or RNA. Thus, according to a further aspect of the invention we provide an

antisense DNA or an antisense RNA of a gene coding for a human metalloproteinase, said gene containing the nucleotide sequence of formulae SEQ I.D. No: 1 or SEQ I.D. No: 2.

The antisense DNA or RNA will correspond to the metalloproteinase gene or a fragment thereof, for example a fragment based on the nucleotide sequence of SEQ I.D. No: 1 or SEQ I.D. No: 2. The antisense DNA or RNA can be produced using conventional means, by standard molecular biology and/or by chemical synthesis as described above. If desired, the antisense DNA and antisense RNA may be chemically modified so as to prevent degradation *in vivo* or to facilitate passage through a cell membrane, and/or a substance capable of inactivating mRNA, for example ribosyme, may be linked thereto, and the invention extends to such constructs.

The antisense DNA or antisense RNA may be of use in the treatment of diseases or disorders in humans in which the over- or unregulated production of the metalloproteinase has been implicated. Such diseases or disorders may include those described under the general headings of infectious diseases, e.g. HIV infection; inflammatory disease/autoimmunity e.g. rheumatoid arthritis, inflammatory bowel disease; osteoarthritis; cancer; allergic/atopic diseases e.g. asthma, eczema; cardiovascular disease e.g. myocardial infarction, congestive heart failure; systemic inflammatory response syndrome e.g. sepsis syndrome; reperfusion injury; malignancy; cachexia; congenital e.g. cystic fibrosis, sickle cell anaemia; dermatologic, e.g. psoriasis, alopecia; neurologic, e.g. multiple sclerosis, migraine headache; renal e.g. uraemia, nephrotic syndrome; obstetric/gynecologic e.g. premature labour, miscarriage, genitourinary prolapse, urinary incontinence, contraception, infertility; transplants e.g. organ transplant rejection, graft-versus-host disease; metabolic/idiopathic disease e.g. diabetes; disorders of the bone such as osteoporosis; and toxicity e.g. due to chemotherapy, cytokine therapy, and anti-CD3 therapy.

The metalloproteinase according to the invention and homologues or fragments thereof may be used to generate substances which selectively bind to the proteins and in so doing regulate the activity of the enzymes.

Such substances include, for example, antibodies, and the invention extends in particular to an antibody which is capable of recognising one or more epitopes on a metalloproteinase containing the amino acid sequence of SEQ I.D. No: 3, or a homologue or fragment thereof. In particular the antibody may be a neutralising antibody

As used herein the term antibody is to be understood to mean a whole antibody or a fragment thereof, for example a $F(ab)_2$, Fab, Fv, V_H or V_K fragment, a single-chain antibody, a multimeric monospecific antibody or fragment thereof, or a bi- or multispecific antibody or fragment thereof.

The antibody according to the invention may be a polyclonal or, especially, a monoclonal antibody. The antibody may belong to any immunoglobulin class, and may be for example an IgG, for example IgG_1, IgG_2, IgG_3 IgG_4, IgE, IgM or IgA antibody. It may be of animal, for example mammalian origin, and may be for example a murine, rat or human antibody. Alternatively, the antibody may be a chimeric antibody. The term chimeric antibody is used herein to mean any antibody containing portions derived from different animal species. Particular examples include those antibodies having a variable region derived from a murine or other antibody constant region, and those antibodies in which one or more CDR sequences and optionally one or more variable region framework amino acids are derived from a murine or other antibody and the remaining portions of the variable and the constant regions are derived from a human immunoglobulin.

Antibodies according to the invention may be prepared by conventional immunisation and recombinant DNA techniques. Thus, for example polyclonal antibodies may be obtained from the sera of animals immunised with a metalloproteinase according to the invention or a homologue or fragment thereof. Any suitable host, for example BALB/c mice where it is desired to obtain a mouse polyclonal antibody, may be injected with the immunogen, the serum collected and the antibody recovered therefrom. Monoclonal antibodies may be obtained from hybridomas derived from the spleen cells of an animal immunised as just discussed and fused to an appropriate "immortal" B-tumour cell. In each

instance, the antibody may be recovered from either the serum or the hybridoma by making use of standard purification and or concentration techniques, for example by chromatography, using for example Protein A or by other affinity chromatography employing a metalloproteinase of the invention or a homologue or fragment thereof.

Once a cell line, for example a hybridoma, expressing an antibody according to the invention has been obtained it is possible to clone therefrom the cDNA and to identify the variable region genes encoding the desired antibody, including the sequences encoding the CDRs. From here, other chimeric antibodies according to the invention may be obtained by preparing one or more replicable expression vectors containing at least the DNA sequence encoding the variable domain of the antibody heavy or light chain and optionally other DNA sequences encoding remaining portions of the heavy and/or light chains as desired, and transforming an appropriate cell line, e.g. a non-producing myeloma cell line, such as a mouse NSO line, in which production of the antibody will occur. In order to obtain efficient transcription and translation, the DNA sequence in each vector should include appropriate regulatory sequences, particularly a promoter and leader sequence operably linked to the variable domain sequence. Particular methods for producing antibodies in this way are generally well known and routinely used. For example, basic molecular biology procedures are described by Maniatis *et al* [Molecular Cloning, Cold Spring Harbor Laboratory, New York, 1989]; DNA sequencing can be performed as described in Sanger *et al* [PNAS 74, 5463, (1977)] and the Amersham International plc sequencing handbook; and site directed mutagenesis can be carried out according to the method of Kramer *et al* [Nucl. Acids Res. 12, 9441, (1984)] and the Anglian Biotechnology Ltd handbook. Additionally, there are numerous publications, including patent specifications, detailing techniques suitable for the preparation of antibodies by manipulation of DNA, creation of expression vectors and transformation of appropriate cells, for example as reviewed by Mountain A and Adair, J R in Biotechnology and Genetic Engineering Reviews [ed. Tombs, M P, 10, Chapter 1, 1992, Intercept, Andover, UK] and in International Patent Specification No. WO 91/09967.

Antibodies and other selective binding agents according to the invention may be of use in therapy, either alone or as a delivery agent, for the delivery of a drug, prodrug, radiometal or radioisotope for example in the treatment of diseases such as those described above in humans and/or other animals, or may find a use as purification agents in the preparation of the human metalloproteinase or homologues or fragments thereof.

In a further use according to the invention, selective binding agents of the invention, such as antibodies, may form the basis of a diagnostic assay to detect the presence or absence in a biological sample (e.g. serum, synovial fluid or a tissue extract) of a metalloproteinase as described herein. Thus for example the binding agent may be brought into contact with a sample of serum, synovial fluid or tissue under conditions in which a complex is formed between the binding agent and target metalloproteinase. Qualitative and/or quantitative detection of the complex can then be used to determine the presence or absence of the metalloproteinase and in particular whether the enzyme is present in an abnormal quantity associated for example with a particular disease state.

The metalloproteinases according to the invention may in particular be used to screen for compounds which regulate the activity of the enzymes and the invention extends to such a screen and to the use of compounds obtainable therefrom to regulate metalloproteinases *in vivo*.

Thus according to a further aspect of the invention we provide a process for obtaining a compound capable of regulating the action of a human metalloproteinase *in vivo* which comprises subjecting one or more test compounds to a screen comprising (A) a metalloproteinase containing the amino acid sequence of SEQ I.D. No: 3 or a homologue or fragment thereof, or (B) a host cell transformed to be capable of expressing a metalloproteinase gene or cDNA or a homologue or fragment thereof containing a nucleotide sequence of SEQ I.D. No: 1 or SEQ I.D. No: 2 or a homologue or fragment thereof.

The screen according the invention may be operated using conventional procedures, for example by bringing the test compound or compounds to

be screened and an appropriate substrate into contact with the metalloproteinase or a cell capable of producing it and determining affinity for the protein in accordance with standard practice.

Any compound obtainable in this way may have a potential use in the treatment in humans and/or other animals of one or more of the above mentioned diseases. The invention thus extends to a compound selected through its ability to regulate the activity of a metalloproteinase *in vivo* as primarily determined in a screening assay utilising a metalloproteinase containing an amino acid sequence of SEQ I.D. No: 3 or a homologue or fragment thereof or a gene coding therefor, for use in the treatment of a disease in which the over- or under-activity or unregulated activity of the metalloproteinase is implicated.

CLAIMS

1. Isolated human DNA comprising the nucleotide sequence of SEQ I.D. No: 1 herein and homologues and fragments thereof.

2. Isolated DNA according to Claim 1 having the human metalloproteinase-encoding nucleotide sequence of SEQ I.D. No: 2 herein and homologues and fragments thereof.

3. An isolated gene or cDNA coding for a human metalloproteinase, said gene or cDNA containing the nucleotide sequence of SEQ ID. No: 1 or SEQ I.D. No: 2 herein or a homologue thereof.

4. An antisense DNA or an antisense RNA of a gene coding for a human metalloproteinase, said gene containing the nucleotide sequence of SEQ I.D. No: 1 or SEQ I.D. No: 2 herein.

5. An isolated human metalloproteinase which contains the amino acid sequence of SEQ I.D. No: 3 and homologues and fragments thereof.

6. An antibody which is capable of recognising one or more epitopes of a metalloproteinase according to Claim 5 or a homologue or fragment thereof.

7. A process for obtaining a compound capable of regulating the action of a human metalloproteinase *in vivo* which comprises subjecting one or more compounds to a screen comprising a metalloproteinase according to Claim 5 or a homologue or fragment thereof.

8. A process for obtaining a compound capable of regulating a human metalloproteinase *in vivo* which comprises subjecting one or more test compounds to a screen comprising a host cell transformed to be capable of expressing a metalloproteinase gene or cDNA containing a nucleotide sequence according to Claims 1 or 2 or a homologue thereof.

9. A compound selected through its ability to regulate the activity of a metalloproteinase *in vivo* as primarily determined in a screening assay utilising a metalloproteinase having an amino acid sequence of SEQ I.D. No: 3 or a homologue or fragment thereof or a gene coding therefor, for use in the treatment of a disease in which the over- or under-activity or unregulated activity of the metalloproteinase is implicated.

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(71) Applicant *(for all designated States except US)*: CELLTECH THERAPEUTICS LIMITED [GB/GB]; 216 Bath Road, Slough, Berkshire SL1 4EN (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: DOCHERTY, Andrew, James, Penrose [GB/GB]; Piccards Cottage, Sandy Lane, Guildford, Surrey GU3 1HF (GB). SLOCOMBE, Patrick, Marcel [GB/GB]; 16 Roman Way, Warfield, Bracknell, Berkshire RG42 7UT (GB).

(74) Agent: HALLYBONE, Huw, George; Carpmaels & Ransford, 43 Bloomsbury Square, London, WC1A 2RA (GB).

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(57) Abstract

DNA sequences coding for a human metalloproteinase are described together with the corresponding antisense DNA and RNA. The DNA may be used to produce the metalloproteinase which may be used to generate antibodies thereto and to obtain other compounds capable of regulating the action of the metalloproteinase *in vivo*.

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Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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[X] Further documents are listed in the continuation of box C.

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